REMARKS

Claim 1 has been amended by limiting the first DW-ACP with the general formula I described in claim 6, and the limitation of the regulator portion described in claim 17 has also been incorporated into claim 1. Accordingly, claim 6 has been cancelled and amended claim 17 is marked herein as withdrawn for being directed to an unelected invention. Support for this amendment can be found throughout the specification. Thus, no new matter is added by this amendment.

In addition, claim 30 has been cancelled to overcome the rejection under 35 USC § 112.

The preceding amendments and the following remarks are believed to be fully responsive to the outstanding Office Action and are believed to place the application in condition for allowance. The Examiner is respectfully requested to reconsider and withdraw the rejections in view of the amendments and remarks as set forth hereinbelow.

I. Rejection under 35 USC § 103

With regard to the rejection under 35 USC § 103, the Examiner has alleged that the present claims 1-5 and 13 are unpatentable over Stone & Wharton (1994, Nucleic Acids Research vol. 22, no. 13, pages 2612-2618), Welsh & McClelland (1990, Nucleic Acids Research vol. 18, no. 24, pages 7213-7218), and Brenner (US Patent No. 5,962,228). With regard to claim 6, the Examiner has alleged that this claim is unpatentable over Stone & Wharton, Welsh & McClelland, Brenner, and Liu and Whittier.

To rebut the assertions of the Examiner, claim 1 has been amended by limiting the first degenerate DW-ACP with the general formula I, as described in claim 6, and the limitation of the

regulator portion described in claim 17 has also been incorporated into claim 1.

The Examiner states in the rejection reason that Stone & Wharton, Welsh & McClelland, and Brenner teach the method described in claim 1 and, in addition to this, that Liu and Whittier teach the AD (arbitrary degenerate) primer which meets the limitations for DW-ACP of general formula I in claim 6.

The Applicant disagrees with the Examiner's interpretation that AD primer meets the limitation of DW-ACP of general formula I.

AD 3 primer is 5'-CA(A/T)CGICNGAIA(G/C)GAA-3'. The first DW-ACP of the present invention is expressed as general formula I of 5'-Xp-Yq-Zr-Qs-3' (I), wherein Xp represents a 5'-end portion having a pre-selected nucleotide sequence, Yq represents a regulator portion comprising at least two contiguous universal or nondiscriminatory bases, Zr represents degenerate random sequence portion having a degenerated random nucleotide sequence, and Qs represents a 3'-end portion having a hybridizing nucleotide sequence substantially complementary to a site on said unknown nucleotide sequence to hybridize therewith.

The Examiner also states that the AD3 and the AD4 primers meet the requirement of the first DW-ACP of the present invention. However, the Examiner's interpretation is erroneous in view of the following points.

First, the first degenerate DW-ACP of the present claim 1 has the unique structure of 5'-Xp-Yq-Zr-Qs-3', that is, the respective portions of Xp, Yq, Zr, and Qs in the primer have the order of 5'-Xp-Yq-Zr-Qs-3'. More specifically, the regulator portion Yq follows the 5'-end portion Xp, the degenerate random sequence portion Zr follows Yq, and 3'-end portion Qs follows Zr. On the contrary, in the AD3 and AD4 primer, N (corresponding to the degenerate

random sequence portion Zr) is located between two residues of I (corresponding to the regulator portion Yq). Thus, it is clearly evident that the structure of the present DW-ACP is totally different from those of AD3 and AD4 primers.

Second, the regulator portion Yq of the present DW-ACP comprises at least two contiguous universal base or non-discriminatory base analog residues. On the contrary to this, the AD3 or AD4 primer of Liu and Whittier has two inosine residues separated by 4-5 other nucleotides, and thus does not comprise contiguous nucleotide having at least two universal base or non-discriminatory base analog residues.

From the view of the unique structure of the first degenerate DW-ACP of general formula I, it is clear that Liu and Whittier do not teach the use of the first DW-ACP.

With regard to claim 17, which requires that the Yq portion of the DW-ACP comprises at least two contiguous universal base or non-discriminatory base analog residues, the Examiner indicates that Brenner renders this feature obvious by teaching primers with contiguous nucleotides having universal base or non-discriminatory base analog residues.

However, it is noteworthy that the location of the contiguous inosine residues in Brenner is at the 5'-end of the primer (as also indicated by the Examiner). Therefore, Brenner does not disclose the specific structure of DW-ACP of 5'-Xp-Yq-Zr-Qs-3'.

The Examiner asserted that an ordinary person skilled in the art would place the inosines at any desired location to maximize the desired results. Further, the Examiner regarded the change of the location of the stretch of inosines as routine optimization.

The Applicant traverses the Examiner's assertion.

The Yq regulator region, comprising at least two contiguous universal base or non-

discriminatory base analog residues, is located between Xp (5'-end portion) and Zr (degenerate random sequence region). The regulator portion Yq prevents annealing of the 5'-end portion sequence (Xp) to a template and facilitates hybridization of degenerate random sequence portion (Zr) and 3'-end portion (Qs) to the template and, thus, restricts the annealing portion of the primer to its degenerate random sequence portion (Zr) and 3'-end portion (Qs). Consequently, the regulator portion (Yq) dramatically improves annealing specificity of the degenerate sequence portion (Zr) plus the 3'-end portion of the first DW-ACP to the template (see paragraphs [0034] and [0111] of the present specification; also see Hwang et al., BioTechniques 35(6):1180-1184, 2003 (a copy is enclosed)).

M.P.E.P. § 2141(II) states "Objective evidence relevant to the issue of obviousness must be evaluated by Office personnel. *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966) at 17-18, 148 USPQ at 467. Such evidence, sometimes referred to as "secondary considerations," may include evidence of commercial success, long-felt but unsolved needs, failure of others, and **unexpected results**" (emphasis added).

As indicated by the Examiner, Brenner explains that deoxyinosine residues are added to balance the annealing and melting temperature of the T primers. Thus, it is clear that the function of deoxyinosine residues in the T primer is only to provide proper annealing and melting temperatures of T primer. On the contrary, the regulator portion Yq of the first degenerate DW-ACP of this invention improves annealing specificity of the Zr (degenerate random sequence portion) plus Qs (3'-end portion) by **unexpected function** of promoting the annealing of Zr to the Qs portion and inhibiting the hybridization of the Xp portion. This unexpected function of the first degenerate DW-ACP comes from the unique structure of the first degenerate DW-ACP

in which the regulator portion (Yq) comprising at least two contiguous nucleotides having universal base or non-discriminatory base analoge residue is located between 5'-end portion (Xp) and degenerate random sequence portion (Zr). Thus, the present method according to claim 1 using the first DW-ACP provides results that **cannot be expected** from the disclosures of the prior art.

The prior art genome walking methods have high background problems due to non-specific priming of DNA walking (DW) primer to templates during PCR. However, the method of present claim 1 using the first DW-ACP can fundamentally eliminate the background problems, which are the major bottleneck of the conventional DW primer.

The objective evidence of the unexpected results of the present method is shown, for example, in Example 1. In Example 1, the effect of the ACP system in DNA walking ACP-PCR was evaluated by comparing ACP system with a conventional longer primer system. The conventional DNA walking longer primer (DW-Ps) does not have poly deoxyinosine residues. The results from the experiments in Example 1 reveal that the conventional DW primer generates many non-specific products during the primary amplification due to the non-specific priming of the DW primer to the template. In contrast, the first DW-ACP binds to the specific sites of the template during the one cycle of the primary amplification (first-stage PCR) under such low stringent conditions, but no longer acts as a primer during the subsequent cycles of the primary amplification (second-stage PCR), which takes place under such high stringent conditions that the annealing portion (i.e., the degenerate sequence and 3'-end portions) of the primer cannot bind to the template or the first DW-ACP primer extension products, and thus the second DW-ACP alone cannot make any product during the secondary PCR amplification.

The results shown in Figure 2 demonstrate that the present DW-ACP system solves the non-specific background problem.

In summary, although the T primer of Brenner has contiguous deoxyinosine residues at the 5'-terminus, the unique structure of the first degenerate DW-ACP of this invention is not taught by Brenner, nor by Stone & Wharton, Welsh & McClelland, and Liu & Whittier. Therefore, the method of amended claim 1 would not have been obvious to one of ordinary person skilled in the art through the combining of the teachings of the cited references.

Furthermore, the present method according to claim 1 using the DW-ACP with unique structure of 5'-Xp-Yq-Zr-Qs-3' produced the unexpected results of the elimination of the non-specific background problem in the DNA walking PCR. The unexpected results are based on the unique structure of regulator portion Yq positioned between Xp (5'-end portion) and Zr (degenerate random sequence portion).

Combining the AD3 and AD4 primer disclosed by Liu and Whittier and the T primer having contiguous 8 deoxyinosine residues at 5'-end of the primer disclosed by Brenner does not render the claimed method obvious. Nor does the prior art predict the specificity of the DNA walking PCR primer binding of the claimed method.

The Examiner pointed out that a skilled person would expect that Tm of the resulting primer containing a contiguous stretch of inosines to be lower compared to the primer containing no inosine. However, contiguous deoxyinosines in the regulator portion Yq of the present DW-ACP prevent the annealing of 5'-end portion Xp to the template DNA, and promote the hybridization of degenerate random sequence portion Zr and 3'-end portion Qs to the template DNA, thereby improving the specificity of DW-ACP to the arbitrary template. This effect cannot

be expected from the mere fact that inosine residues lower the annealing temperature of the primer. Accordingly, the Examiner's indication of obviousness of this invention on the basis of routine optimization is rebutted.

In addition, since amended claim 1 is not obvious over Stone & Wharton, Welsh & McClelland, Brenner, and Liu & Whittier, the dependent claims 2-5, 7, 9-11, 13-16, and 18-22 of claim 1 also are patentable over these references.

Consequently, the Applicant respectfully requests that this rejection be withdrawn.

II. Rejection under 35 USC § 112

The Examiner rejected claim 30 because this claim recited use without setting forth any steps involved in the process.

The Applicant cancels claim 30. Accordingly, the Applicant respectfully requests that this rejection be withdrawn.

CONCLUSION

With regard to the rejection under 35 USC § 103, the prior art does not teach or suggest the method according to the amended claim 1 using DW-ACP having unique structure. In addition, the method according to claim 1 provides unexpected improvements of annealing specificity compared to conventional DNA walking PCR method. Accordingly, it is clear that the teachings of Stone & Wharton, Welsh & McClelland, Brenner, and Liu & Whittier do not teach or suggest the improvement of the annealing specificity of DNA walking primer by positioning the regulator portion (Yq) comprising at least two contiguous deoxyinosine residues between 5'-end portion (Xp) and degenerate random sequence portion (Zr).

With respect to the rejection under 35 USC § 112, claim 30 has been cancelled.

Therefore, in view of the foregoing remarks, the Applicant respectfully requests reconsideration of the present claims and the timely allowance of the claims.

Enclosed is a petition to extend the period for replying for three months, to and including April 1, 2009. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Susan M. Michaud, Ph.D.
Reg. No. 42,885

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045